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Variation in photosynthetic components among photosynthetically diverse cotton genotypes

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Abstract

Photosynthesis is an important component of upland cotton (*Gossypium hirsutum* L.) yield, but little has been done to increase the photosynthetic performance within the cotton germplasm pool. Part of this dilemma is due to the multi-component aspect of this process and also to lack of information on genetic variation among such components. The objectives of this research were to identify genetic variability in photosynthetic components for six cotton genotypes previously shown to differ in leaf CO_2-exchange rates (CER) and to determine if an afternoon decline in photosynthesis altered genotypic differences in CER. CO_2-exchange rates were measured at several internal CO_2 levels (C_i) to generate CER vs. C_i curves for each genotype and thereby isolate some of the components of photosynthesis. Ribulose 1,5 bisphosphate carboxylase-oxygenase (Rubisco), hydroxypyruvate reductase, malate dehydrogenase, and catalase activities were assayed on leaves used to generate the CER vs. C_i curves. Ambient CER and chlorophyll (Chl) fluorescence measurements were taken before and after solar noon to test for an afternoon decline in photosynthesis. Dixie King, a low ambient CER genotype, exhibited a greater CO_2 compensation point, lower carboxylation efficiency, and reduced Photosystem II (PS II) activity than the other genotypes. The carboxylation efficiency of DES 119 was 13% greater than STV 508 and 29% greater than Dixie King, but not different from the other genotypes in 1994. Pee Dee 3 had greater maximum assimilation rate (*A*) than all other genotypes except STV 213 in 1993. Although no significant genotype by time of day interaction was detected, CER and Chl fluorescence variable to maximum ratio (Fv/Fm) were reduced 8% and 39%, respectively, in the afternoon as compared to the morning. This study demonstrates genetic variations in many of the components of photosynthesis. However, the narrow range of variation in such components for superior photosynthesizing genotypes explains why difficulties are encountered when breeding for increased photosynthesis.

Abbreviations: A – maximum CO_2-exchange rate at high internal CO_2 concentrations; C_i – internal CO_2 concentration; CER – CO_2-exchange rate; Chl – chlorophyll; CO_2 comp. – CO_2 compensation point; Fv/Fm – variable fluorescence/maximal fluorescence ratio; g_s – stomatal conductance to CO_2; λ – midpoint in the rise of the CER vs. C_i curve; HPR – hydroxypyruvate reductase; PPFD – photosynthetic photon flux density; RuBP – ribulose 1,5 bisphosphate; Rubisco – ribulose 1,5 bisphosphate carboxylase-oxygenase; SLW – specific leaf weight; WUE – water use efficiency

Introduction

Photosynthesis is a principle component of crop yield and yet little has been accomplished genetically to intentionally and simultaneously advance both photosynthesis and yield within new germplasm of any crop. In both upland cotton (*Gossypium hirsutum* L.) and Pima cotton (*Gossypium barbadense*) genetic diversity has been detected in leaf CO_2-exchange rates (CER) with the higher yielding genotypes exhibiting superior leaf CER (Cornish et al. 1991; Pettigrew and Meredith 1994). However, these genotypes were bred

only with increased yield as the primary goal. The increased leaf photosynthesis was an unintentional but beneficial secondary consequence of these breeding programs.

Part of the problem in trying to genetically improve photosynthesis as a venue toward yield increases is that photosynthesis is a highly complex, multi-step process with many points of regulation. Improvement in one step may prove fruitless if another component remains rate limiting for the overall process. To successfully advance both photosynthesis and yield through a cotton breeding program, adequate genetic variation in most photosynthetic components is probably needed. Hopefully, these components could then be combined in the most optimum configuration for enhanced photosynthesis, which would then need to be coupled with optimal partitioning of the photosynthate into reproductive growth.

Tools exist to nondestructively estimate *in vivo* activities of some the photosynthetic system components. Chlorophyll (Chl) fluorescence trace measurements can be used to calculate the ratio of variable fluorescence (Fv) to maximal fluorescence (Fm), [Fv/Fm], which is proportional to the quantum yield of photochemistry and is therefore an estimate of Photosystem II (PS II) efficiency (Krause and Weis 1991). By plotting the CER *versus* the internal CO_2 concentration (C_i) over a range of C_i levels, one can derive CO_2 compensation points (estimates of photorespiration), the maximum assimilation rate at high C_i (indications of efficiency in chloroplast electron transport processes and regeneration of ribulose 1,5 bisphosphate), and the initial slope of the curve (estimates of carboxylation efficiency) (von Caemmerer and Farquhar 1981). When combining these non-destructive methods with destructive techniques, such as traditional *in vitro* biochemical assays for enzymatic activity, one can probe for regulatory points in the system and for possible variations in that regulation. Thus far, however, little information exists documenting genotypic variations in any of the photosynthetic components for cotton.

Genotypic leaf differences documented for cotton include specific leaf weight (SLW), Chl concentration, and protein concentration (Wells et al. 1986; Cornish et al. 1991; Pettigrew et al. 1993; Pettigrew and Meredith 1994). Most of these leaf differences can be attributed to variations in leaf thickness or density, as exhibited by SLW differences and are often correlated with higher CER per unit leaf area (Bhagsari and Brown 1968; Morgan and LeCain 1991; Pettigrew et al. 1993; Pettigrew and Meredith 1994). While Benedict et al. (1981) were able to demonstrate 2-fold differences in ribulose-1, 5-bisphosphate carboxylase-oxygenase (Rubisco) activity between *G. hirsutum* (AD_1) and *G. davidsonii* (D_{3-d}), little work has been devoted to screening for genotypic differences in Rubisco concentration or activity within the *G. hirsutum* normal leaf type germplasm. In addition, little work has been performed on the other enzymes involved in the photosynthetic process for cotton.

Genetic factors can sometimes be overwhelmed by climatic or environmental influences experienced during growth. One prime example is the decline in CER during the afternoon (compared to morning rates when measured at comparable light intensities). These lower photosynthetic rates in the afternoon have been attributed to photoinhibition (Powles 1984), carbohydrate feedback inhibition (Nafziger and Koller 1976; Peet and Kramer 1980), high temperature stress (Baldocchi et al. 1981; Perry et al. 1983), transient water stress (Sharkey 1984), and stomatal closure due to increasing vapor pressure deficit (VPD) (Farquhar et al. 1980; Bunce 1982 and 1983; Pettigrew et al. 1990). In Pima cotton, Cornish et al. (1991) demonstrated that genotypic differences in leaf CER observed in the morning disappeared during the afternoon hours. Genotypes that are able to maintain their photosynthesis during the afternoon should have more photosynthate for growth and yield development than other genotypes that make less efficient use of the afternoon sun. Identifying components limiting photosynthesis during the afternoon could aid in breeding plants that use the afternoon sunlight more efficiently.

Before the simultaneous breeding for increased yield and increased photosynthesis becomes routinely successful, adequate genetic variation in components of the photosynthetic process must first be identified. The first objective of this research was to identify genetic variability in photosynthetic components among cotton genotypes by measuring CER over a range of C_i, by using Chl fluorescence technology to assay for PS II efficiency, and by assaying for the activities of various photosynthetic and photorespiratory enzymes. The second objective was to determine whether the afternoon photosynthetic decline altered the expression of genotypic differences in CER and Chl fluorescence.

Materials and methods

Field studies were conducted in 1993 and 1994 on a Bosket fine sandy loam (fine-loamy, mixed, thermic mollic Hapludalfs) near Stoneville, MS, using six upland cotton genotypes previously shown to differ in leaf CER (Pettigrew and Meredith, 1994). Genotypes used in this study were 'DES 119', 'MD 51 ne', 'Stv. 213', 'Pee Dee 3', 'Dixie King', and 'Stv. 508'. Plots, which were comprised of 4 rows 6.1-m-long and spaced 102 cm apart, were planted 28 April 1993 and 22 April 1994. These plots were originally overseeded and then, at the first or second true leaf stage, thinned to one plant per 15 cm of row for a final population density of 65 000 plants ha^{-1}. Prior to planting each year, 110 kg ha^{-1} N was incorporated into the soil. Conventional weed and insect control measures were utilized as needed each year. To minimize the effects of moisture stress, the plots were furrow-irrigated when needed each year. Experimental design used was a randomized complete block with six replications.

Ambient CO_2 gas exchange measurements were taken on the youngest fully expanded, disease-free, fully sunlit leaves in each plot. Measurements were made with the leaves oriented perpendicular to the sun using a LI-COR LI-6200 portable photosynthesis system (Li-Cor, Lincoln, NE[1]). The gas exchange measurements were made on two leaves per plot with the average of the two leaves used for later statistical analysis. Photosynthetic photon flux densities (PPFD) reaching the adaxial leaf surfaces during the measurements were $\geq$ 1600 at all times. These ambient CER measurements were taken first before solar noon and then again after solar noon on the same day. The two leaves measured before solar noon were tagged and then measured again during the after solar noon measurements on the same day. These data were collected on 29 June, 30 June, 26 July, and 27 July in 1993. In 1994, the data were taken on 5 July and 6 July.

Chlorophyll fluorescence measurements were taken on the same leaves and at the same time as the ambient CER measurements using a CF-1000 Chl fluorescence measurement system (P.K. Morgan, Inc., Andover, MA). Following the gas exchange measurement, a dark adaption cuvette was placed on the leaf and it was allowed to dark adapt for at least 15 min prior to the fluorescence measurement. Ninty seconds were used in taking each measurement. Similar to the gas exchange measurements, measurements were taken on two leaves per plot, both before and after solar noon, with the average of the two leaves used in later statistical analysis of the Chl flourescence measurements.

Photosynthetic CO_2 response curves were generated by elevating the CO_2 concentration in a tripod mounted, one L leaf chamber to at least 700 μl l^{-1} and then monitoring CER, stomatal conductance (g_s) and C_i as the CO_2 concentration inside the chamber declined. Methods employed for generation of the CO_2 response curves were similar to those described by McDermitt et al. (1989) and Faver et al. (1996). Measurements were collected on one leaf per plot with the leaf oriented perpendicular to the sun and the PPFD level reaching the adaxial leaf surface $\geq$ 1600. All measurements were completed prior to solar noon and took approximately 15 min per measurement. A tripod mounted fan (for the external ventilation of the leaf chamber) and a 60 cm $\times$ 60 cm $\times$ 1 cm clear plexiglass shield filled with water and positioned above the leaf chamber (for absorption of incident infared radiation) were utilized to maintain the leaf temperature at a relatively consistent level throughout the long duration of this measurement. Following completion of the measurement, three 0.4-cm^2 leaf disk were cut from the leaf for Chl concentration measurements. The remainder of the leaf was instantly frozen with liquid N and transported to the laboratory. Once in the laboratory, the frozen leaves were ground in liquid N and then stored at -85 °C for later enzymatic assays.

Chlorophyll was extracted from the leaf disks with 5 ml of 950 ml l^{-1} ethanol over a 24 h period of darkness at 30 °C as described previously (Pettigrew and Meredith 1994). The Chl concentration of the extract was quantified spectrophotometrically as described by Holden (1976).

Rubisco (EC 4.1.1.39) was extracted from the ground leaf material with an extraction buffer containing 100 mM Bicine (pH 7.8), 5 mM $MgCl_2$, 1 mM Na_2EDTA, 15 mM 2-mercaptoethanol, 5 mM dithiothreitol, 1 ml l^{-1} Triton X-100, and 15 g l^{-1} polyvinylpyrrolidone. The leaf material was kept in vials in liquid N until just before the assay. To minimize secondary phenolic compounds from being released from the glandular structures found throughout cotton tissue and thereby inhibiting the activities of the enzymes, an extensive homogenization of the tissue was not performed. For the extraction, a small aliquot

[1] Trade names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product or service, and the use of the name by UDSA implies no approval of the product or service to the exclusion of others that may also be suitable.

(approximately 0.075 g) of ground leaf material was placed in a previously weighed microfuge tube containing 1 ml of the extraction buffer. The microfuge tube was reweighed, shaken rapidly for a very brief period of time (15 s) to suspend the leaf material, then centrifuged at 12 300 *g* for 30 s to pellet the leaf material. The assay for initial activity was started by adding 50 μl of the supernatant to one mL of assay buffer containing 50 mM Bicine (pH 8.0), 15 mM $MgCl_2$, 1 mM Na_2EDTA, 10 mM NaCl, 10 mM $NaHCO_3$, 5 mM dithiothreitol, 5 mM phosphocreatine, 5 mM adenosine triphosphate (ATP), 500 μM ribulose 1,5 bisphosphate (RuBP), 120 μM nicotinamide adenine dinucleotide (NADH), 7000 units l^{-1} glyceraldehyde 3-phosphate dehydrogenase, 7000 units l^{-1} phosphoglycerate kinase, and 7000 units l^{-1} creatine phosphokinase. The carboxylation rate was determined by monitoring the oxidation rate of NADH at 340 nm and assuming two molecules of NADH oxidized per molecule of CO_2 fixed. Total activity was determined by adding 50 μl of supernatant to one ml of assay buffer without the RuBP, allowing mixture to sit for 10 min while the Rubisco activated, and then adding the RuBP and monitoring the NADH oxidation rate. Chlorophyll determination was performed on 0.4 ml of the extract supernatant as previously described.

Three other enzymes, malate dehydrogenase (EC 1.1.1.37), hydroxypyruvate reductase (EC 1.1.1.81) and catalase (EC 1.11.1.6), were extracted as above except the extraction buffer contained 100 mM potassium phosphate (pH 7.2), 1 mM EDTA, 1 mM iodoacetamide, 1 mM benzamidine, 1 ml l^{-1} Triton X-100. Before use, 10 g l^{-1} polyvinylpyrrolidone was added to the buffer solution as an additional enzyme protectant. Enzyme activities were assayed spectrophotometrically at 24 °C. The enzymes were assayed according to published protocols: malate dehydrogenase according to Tolbert et al. (1968); hydroxypyruvate reductase according to Kohn and Warren (1970); and catalase according to Kunce and Trelease (1986). All activities were measured for a few minutes in 1 ml assay volumes.

Each year, the Weibull model (Ratkowsky 1983) was used to fit the data trend of the CER vs. C_i measurements for each genotype from all six reps to a non-linear equation of the form CER = A * (1 − exp ((−1/λ)(C_i − CO_2 comp.))). In this equation, A = maximum assimilation rate, λ = the C_i level corresponding to the halfway point in the rise of the curve, and CO_2 comp. = the C_i level where CER is zero (CO_2 compensation point). This non-linear equation was chosen because it gave the best fit of the data. The equations and asymptotic 95% confidence intervals of the equation components were obtained from the PROC NLIN function of SAS (SAS Institute, Cary, NC). Genotypic differences in the equations describing the CER vs. C_i trend were separated by F-tests (Hinds and Milliken 1987). The initial slope of the CER vs. C_i curve was determined by taking the CER and C_i data when $C_i \leq 100$ μl l^{-1} and fitting them to linear regression equations. Ambient CO_2 gas exchange measurements, Chl fluorescence measurements, SLW, Chl concentration, enzyme activities, and CER vs. C_i initial slope data were analyzed by analysis of variance. Means were separated via an $LSD_{0.05}$.

Results and discussion

Leaf CER differed among the genotypes each year of the study, but the differences were similar for each year (Table 1). The same genotypic differences in gas exchange previously described by Pettigrew and Meredith (1994) were again manifest in this study. MD 51 ne and DES 119 appeared to consistently have higher CER while Dixie King and Stv. 508 appeared to consistently have lower CER. Stomatal conductance to CO_2 did not differ among genotypes in 1993, but in 1994, DES 119 had a lower g_s and MD 51 ne had a greater g_s than the other genotypes. The high CER and low g_s of DES 119 produced the lowest C_i and highest water use efficiency (WUE) in both 1993 and 1994. These genotypic differences provided the background justification for investigating possible genetic differences in the photosynthetic response to different CO_2 levels.

Genotypic differences were not previously detected for the ratio of variable to maximal chlorophyll fluorescence (Fv/Fm) (Pettigrew and Meredith 1994) using these genotypes. In this study however, Dixie King had 24% lower Fv/Fm than that of the next lowest genotype, MD 51 ne, in 1993 (Table 1). None of the other genotypes differed for Fv/Fm that year. In 1994, the Fv/Fm of Dixie King was numerically but not significantly lower than that of the other genotypes.

The variation in CER among genotypes to changes in the C_i level was investigated in both 1993 and 1994. Figure 1 illustrates the response of MD 51 ne in 1994 to changes in C_i. There was rep to rep variability in the response, but statistically it was appropriate to pool all replications together and generate one equation for each genotype, each year. Although this figure

Table 1. Gas exchange parameters and chlorophyll fluorescence variable to maximal ratio averaged over time of day for 1993 and 1994

Genotype	CER	Stomatal conductance	C_i	WUE	Fv/Fm
	μmol m^{-2} s^{-1}	mol m^{-2} s^{-1}	μl l^{-1}	mol CO_2mol H_2O^{-1}	
1993					
DES 119	31.3	0.56	270	2.14	0.401
MD 51 ne	31.0	0.60	274	2.10	0.399
STV 213	30.5	0.54	272	2.14	0.400
Pee Dee 3	30.2	0.58	276	2.04	0.464
STV 508	29.3	0.56	275	2.02	0.406
Dixie King	28.5	0.56	276	1.94	0.303
LSD 0.05	1.2	NS[a]	4	0.09	0.084
P > F	0.01	0.25	0.03	0.01	0.02
1994					
DES 119	31.6	0.60	268	1.92	0.615
MD 51 ne	33.1	0.70	272	1.86	0.565
STV 213	32.1	0.67	272	1.86	0.566
Pee Dee 3	31.6	0.66	272	1.83	0.561
STV 508	29.8	0.64	275	1.73	0.523
Dixie King	30.1	0.65	276	1.72	0.504
LSD 0.05	1.4	0.03	5	0.08	NS
P > F	0.01	0.01	0.02	0.01	0.17

[a] NS = not significantly different at $P \leq 0.05$.

Table 2. CER vs. C_i components from the equation
$\text{CER} = A^*(1 - \exp((-1/\lambda)(C_i - CO_2\text{comp.})))$[a]

Year	Genotype	A	CO_2 Compensation point	λ
		μmol m^{-2} s^{-1}	μl l^{-1}	
1993				
a[b]	DES 119	52 (50 – 55) [c]	217 (194 – 239)	64 (62 – 67)
	Dixie King	50 (47 – 52)	218 (200 – 236)	69 (67 – 71)
b	MD 51 ne	50 (49 – 52)	207 (202 – 232)	63 (62 – 65)
	Pee Dee 3	57 (55 – 59)	255 (235 – 274)	65 (63 – 67)
a	STV 213	55 (53 – 58)	241 (218 – 265)	63 (60 – 65)
b	STV 508	50 (48 – 52)	215 (195 – 235)	66 (63 – 68)
1994				
a	DES 119	52 (47 – 56)	289 (247 – 332)	62 (58 – 67)
	Dixie King	48 (42 – 54)	338 (261 – 416)	66 (57 – 74)
a	MD 51 ne	55 (48 – 63)	319 (245 – 393)	64 (57 – 70)
b	Pee Dee 3	57 (47 – 67)	399 (287 – 512)	63 (56 – 71)
c	STV 213	47 (42 – 52)	265 (206 – 324)	66 (57 – 74)
bc	STV 508	50 (45 – 55)	307 (249 – 364)	67 (62 – 73)

[a] '*A*' = estimate of the maximum CER, 'λ' = halfway point in the rise of the curve, and 'CO_2 comp.' = C_i level where CER is zero.
[b] Genotypes within each year with similar letters do not differ in CER vs. C_i curves, otherwise, the equations are significantly different among genotypes.
[c] Numbers in the parentheses are asymptotic 95% confidence intervals.

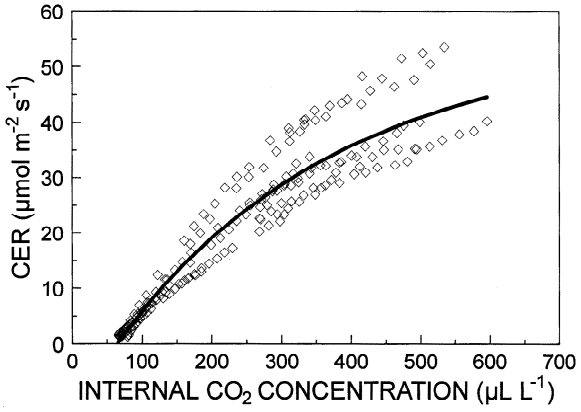

Figure 1. CO_2-exchange rate (CER) at various internal CO_2 concentrations (C_i) for the genotype MD 51 ne in 1994. Data from all six replications were combined in this figure. The CER vs. C_i curve was generated by the equation CER = A * (1−exp((−1/λ)(C_i −CO_2 comp.))). In this equation, A = estimate of the maximum CER, λ = halfway point in the rise of the curve, and CO_2 comp. = C_i level where CER is zero (CO_2 compensation point). Specific values of equation constants for each genotype are provided in Table 2.

is specific for this particular year and genotype combination, it is typical of the response generated by other genotypes for each year. The non-linear equations developed for each genotype to describe the CER vs. C_i response were found to be significantly different among the genotypes each year of the study (Table 2). Both years, the equation for Dixie King was determined to be significantly different than the equation for all the other genotypes. The equation for Pee Dee 3 was also different than all other genotypes in 1993. Equations for the remaining genotypes were determined to be similar to an equation for at least one other genotype in both 1993 and 1994.

Breaking these non-linear equations down and isolating specific components of the equations, we were also able to detect genotypic differences for these components (Table 2). MD 51 ne had a 9% lower CO_2 compensation point (the C_i level where CER is zero) than Dixie King in 1993. None of the other genotypes differed in their CO_2 compensation points that year. The CO_2 compensation point is indicative of the amount of photorespiration that is occurring; thus these 1993 results indicate that Dixie King may exhibit higher photorespiration per unit leaf area. This finding also fits the pattern of Dixie King having a lower leaf CER at ambient CO_2 than all other genotypes except Stv. 508 (Table 1). No genotypic differences in CO_2 compensation points were identified in 1994, but numerically, Dixie King had one of the highest CO_2 compensation points. Maximal CER at high C_i levels (A), an estimate of chloroplast electron transport and RuBP regeneration efficiency (von Caemmerrer and Farquhar 1981), were different among genotypes in 1993. Pee Dee 3 produced a significantly higher A than all other genotypes except for STV 213. The A of STV 213 was significantly different from that of other genotypes, except for Pee Dee 3 and DES 119. None of the other genotypes differed that year. While no significant genotypic differences were detected for A in 1994, Pee Dee 3 again exhibited the highest A, numerically. These results indicate that Pee Dee 3 may have a more efficient system for regeneration of RuBP or electron transport than the other genotypes. The higher Chl Fv/Fm of Pee Dee 3 compared to that of Dixie King in 1993 (Table 1) supports the idea of increased electron transport. Pee Dee 3 also tended to require greater C_i levels to attain comparable high CER of the other genotypes as indicated by its significantly greater λ (the mid-point in the rise of the curve) than all genotypes besides STV 213 in 1993. In 1994, the λ of Pee Dee was statistically greater than only that of STV 213. None of the other genotypes differed statistically in λ for that year. Although no specific biological meaning can be assigned to λ, the findings suggest that some factor other than regeneration of RuBP or electron transport is limiting the CER of Pee Dee 3 at the lower C_i levels. STV 213 demonstrated considerable year to year variation in the CER response to C_i. The other genotypes appeared to be more consistent in performance across the years.

Two estimates of carboxylation efficiencies for the genotypes were obtained in both 1993 and 1994. Carboxylation efficiency was estimated by assays of Rubisco activity and by determining the initial slope of the CER vs. C_i curve (von Caemmer and Farquhar 1981). In 1993, neither method of estimating carboxylation detected differences among the genotypes on a leaf area basis (Table 3). In 1994, however, genotypes differed for both initial and total Rubisco activity ($P \leq 0.10$) and in the initial slope of the CER vs. C_i curve ($P \leq 0.05$). The genotypic trends observed in Rubisco activity were similar between initial and total activities and closely matched the genotypic rankings in CER (Table 1). Trends were that Dixie King had low Rubisco activities while DES 119, STV 213, and MD 51 ne tended to have higher Rubisco activites. DES 119 had a greater carboxylation efficiency, as estimated by the initial slope of the CER vs. C_i curve, than either Dixie King or STV 508, but was not different from the other genotypes (Table 3). The initial slope of the CER

Table 3. Cotton leaf initial and total Rubisco activities and initial slope of CER vs. C_i in 1993 and 1994

Genotype	1993			1994		
	Initial Rubisco activity	Total Rubisco activity	CER vs. C_i initial slope	Initial Rubisco activity	Total Rubisco activity	CER vs. C_i initial slope
	μmol m^{-2} s^{-1}		$\frac{\mu\text{mol m}^{-2}\text{ s}^{-1}}{\mu\text{l l}^{-1}}$	μmol m^{-2} s^{-2}		$\frac{\mu\text{mol m}^{-2}\text{ s}^{-1}}{\mu\text{l l}^{-1}}$
DES 119	84.6	122.2	0.192	66.3	81.5	0.161
Dixie King	77.0	103.5	0.192	50.0	70.3	0.125
MD 51 ne	73.4	99.8	0.193	64.0	87.7	0.155
Pee Dee 3	77.0	108.7	0.194	59.6	74.9	0.143
STV 213	68.8	101.4	0.207	77.0	105.0	0.154
STV 508	69.2	93.8	0.191	60.3	78.4	0.142
LSD 0.05	NS[a]	NS	NS	NS	NS	0.017
P > F	0.76	0.59	0.50	0.07	0.08	0.003

[a] NS = not significantly different at $P \leq 0.05$.

vs. C_i curve for Dixie King was significantly lower than that of all other genotypes, with the exception of STV 508. The genotypic estimates of carboxylation produced by either enzyme assays or initial slope determination were similar and paralleled the ambient CER measurements (Table 1).

Three enzymes, i.e. catalase, hydroxypyruvate reductase, and malate dehydrogenase were assayed to determine activity differences among genotypes. Catalase and hydroxypyruvate reductase are marker enzymes for peroxisomes and are associated with glycolate metabolism during photorespiration. Catalase metabolizes the toxic byproduct, H_2O_2, from photorespiration in leaves. Catalase, therefore, acts as a cellular protectant during photorespiration. Parker and Lea (1983) reported chloroplast destruction in a catalase deficient mutant of barley, whereas, Zelitch (1992) demonstrated decreased photorespiration and increased photosynthesis in a tobacco mutant with increased catalase. MD 51 ne had a significantly greater catalase activity than all other genotypes except for STV 213 in 1993 (Table 4). The other genotypes demonstrated no differences in catalase activity that year. In 1994, no genotypic differences were detected for catalase activity. Similar to catalase, hydroxypyruvate reductase (HPR) is exclusively localized in peroxisomes. Hydroxypyruvate reductase converts hydroxypyruvate (a product of glycolate metabolism) into glycerate which can then be metabolized. Therefore, HPR activity, theoretically, should be correlated with the plants ability to recycle products of photorespiration into beneficial sugars and photosynthesis. Unfortunately, HPR activity did not differ among the genotypes either year of the study. Malate dehydrogenase (NADH dependent) has multiple isoforms in plants (cytoplasmic, mitochondrial, and peroxisomal) and is a key component in plant respiration where it generates oxaloacetate and NADH from malate. MD 51 ne had greater malate dehydrogenase activity than all the genotypes, except for Pee Dee 3 in 1993. Malate dehydrogenase activity was similar among the remaining genotypes in 1993 and did not differ among any of the genotypes in 1994.

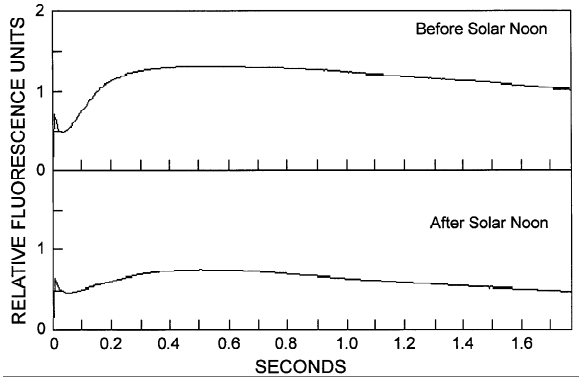

Figure 2. Chlorophyll fluorescence traces, from the same leaf of MD 51 ne, collected before solar noon and after solar noon on the same day in 1994. This figure represents only one replicate of MD 51 ne, but is representative of the Chl fluorescence traces produced by the other replications. Leaves were allowed to dark adapt for at least 15 min prior to initiation of the measurements.

Measurements of ambient CO_2 gas exchange and Chl fluorescence were collected both during the morn-

Table 4. Cotton leaf enzyme activities in 1993 and 1994

Genotype	Catalase	Hydroxypyruvate reductase	Malate dehydrogenase	Chlorophyll concentration
	μkat μg Chl^{-1}[a]	- - - - μmol μg Chl^{-1} min^{-1} - - - -		mg m^{-2}
1993				
DES 119	0.426	0.223	0.841	438
Dixie King	0.364	0.220	0.857	410
MD 51 ne	0.589	0.313	1.180	416
Pee Dee 3	0.428	0.277	0.977	439
STV 213	0.484	0.270	0.928	433
STV 508	0.393	0.228	0.752	410
LSD 0.05	0.142	NS[b]	0.243	NS
P > F	0.04	0.08	0.03	0.53
1994				
DES 119	0.394	0.269	1.331	430
Dixie King	0.301	0.265	1.311	348
MD 51 ne	0.311	0.204	1.115	433
Pee Dee 3	0.248	0.216	1.074	382
STV 213	0.352	0.248	1.228	391
STV 508	0.357	0.237	1.271	353
LSD 0.05	NS	NS	NS	64
P > F	0.08	0.49	0.69	0.04

[a] μkat = μmol s^{-1}.
[b] NS = not significantly different at $P \leq 0.05$.

ing and after solar noon on the same leaves, at similar PPFD intensities, for all genotypes in 1993 and 1994, to determine if genotypic differences detected in the morning prevailed into the afternoon hours. No significant genotype by time of day interaction was detected for any of the gas exchange measurements or fluorescent parameters either year of the study. Although the genotypic differences detected in the morning persisted into the afternoon, the levels measured in the afternoon were considerably lower than the comparable morning rates during both years of the study (Table 5). Averaged across both years, measurements of CER were 8% lower, C_i 4% lower, and WUE 27% lower in the afternoon, compared to morning values. The exception to this was g_s, which was 17% lower during the afternoon in 1993 but did not significantly differ between times of day in 1994. Photosystem II efficiency, as estimated by the Chl fluorescence Fv/Fm ratio and averaged across both years, was 39% lower in the afternoon. Groom et al. (1990) reported a similar Fv/Fm decline during the afternoon for winter wheat (*Triticum aestivum* L.) leaves. In addition to lower Fv/Fm levels, the overall afternoon fluorescence traces for the cotton leaves were altered when compared to the morning traces (Figure 2). Although this figure is for only one plot of MD 51 ne in 1994, it is representative of the morning and afternoon traces produced for other plots and genotypes.

This experiment demonstrated that genetic variability exists in many of the components of the photosynthetic system for cotton. Unfortunately, most but not all of this variability was in the negative direction. Dixie King, which consistently produced a lower CER (Table 1), exhibited a greater CO_2 compensation point (Table 2), indicative of greater photorespiration, than the other genotypes. While other processes besides photorespiration, such as mitochondrial respiration, can contribute to the CO_2 compensation point (Azcon-Bieto et al. 1981), photorespiration, presumably, is still the dominant component determining CO_2 compensation points. The genetic variation in CO_2 compensation points would still be intact even if mitochondrial respiration played a significant role. In addition, PS II activity (as indicated by the Chl fluorescence Fv/Fm ratio (Table 1)) and carboxylation efficiency (as indicated by Rubisco activity and the initial slope of the CER vs. C_i curve (Table 3)) were lower in Dixie King. One of the theoretical

Table 5. Gas exchange parameters and chlorophyll variable: maximal fluorescence ration (Fv/Fm) averaged over genotypes for two years and at two times of day

Time of day	CER	Stomatal conductance	C_i	WUE	Fv/Fm
	μmol m^{-2} s^{-1}	mol m^{-2} s^{-1}	μl l^{-1}	mol CO_2 mol H_2O^{-1}	
1993					
Morning	30.1	0.65	281	2.08	0.545
Afternoon	26.9	0.54	264	1.52	0.246
LSD 0.05	0.5	0.03	4	0.07	0.080
1994					
Morning	32.4	0.66	276	2.11	0.637
Afternoon	30.3	0.65	269	1.54	0.474
LDS 0.05	0.8	NS[a]	4	0.10	0.062

[a] NS = not significantly different at $P \leq 0.05$.

beneficial purposes proposed for photorespiration is to process excess electrons generated by the light harvesting antenna system and thus minimize photoinhibition (Austin 1994). With a lower carboxylation rate, photorespiration for Dixie King may have had to increase to accommodate excess captured light energy. Nevertheless, this increased use of products from the light harvesting-electron transport system by photorespiration did not overcome the carboxylation limitation to photosynthesis and thus the efficiency of PS II to process electrons produced by light harvesting was reduced for Dixie King (Farquhar and Sharkey 1994).

Viewing these genotypic differences in photosynthetic components from a more positive perspective, DES 119 demonstrated a greater initial slope of the CER vs. C_i curve (increased carboxylation efficiency) than STV 508 or Dixie King (Table 3). This increased carboxylation rate per unit leaf area can partially be explained by the increased SLW of DES 119, compared to STV 508 and Dixie King (Pettigrew and Meredith 1994). Faver et al. (1996) had also previously demonstrated differences in the CER vs. C_i initial slope between two cotton genotypes. The results from both studies seem encouraging for detecting differences in the carboxylation rate among cotton genotypes. However, the lack of differences in carboxylation among the high yielding genotypes of our study leads one to question whether there is sufficient genetic diversity for Rubisco carboxylase activity within the *Gossypium hirsutum* germplasm to further improve the carboxylation rate of Rubisco in cotton. Perhaps more genotypes need to be screened for this trait before anything definitive can be said about genetically improving Rubisco activity in cotton.

Pee Dee 3 appears to be more proficient at electron transport and regenerating RuBP than some of the other genotypes (Table 2). Faver et al. (1996) also found that the two genotypes used in their study differed in the ability to regenerate RuBP. The superior photosynthetic genotype in the Faver et al. (1996) study exhibited increased carboxylation efficiency and RuBP regeneration capacity, while the elevated ability to regenerate RuBP exhibited by Pee Dee 3 in our study was not associated with increased carboxylation efficiency. An interesting combination would be to cross Pee Dee 3 (good in regenerating RuBP but average in Rubisco activity) with DES 119 (good in Rubisco activity but average in regenerating RuBP). This hypothetical cross may lead to increased photosynthesis, but, on the other hand, it may only reveal another rate limiting point in the process.

A further limitation to the photosynthetic performance of the crop is caused by the afternoon decline in photosynthesis. From our data, it appears that reductions in both g_s and PS II efficiency contribute to this decline in photosynthetic performance during the afternoon hours (Table 5). Although the PS II efficiency decline might be partially attributed to photoinhibition, Baker and Ort (1992) describe an intrinsic regulatory reduction of PS II activity after exposure to high light intensities, often confused with photoinhibition, that does not cause damage to the photosynthetic apparatus. This process is believed to redirect absorbed photons away from reaction centers and dissipate them as heat instead of damaging the photosynthetic unit.

Most likely, this process also contributed to the afternoon decline in photosynthesis observed in this study. The ability to maintain superior photosynthesis during the afternoon hours would obviously be a desirable trait for any genotype to possess. Unfortunately, all the genotypes in this study exhibited the same relative decline in photosynthesis during the afternoon, as demonstrated by the lack of a significant genotype by time of day interaction for CER (data not shown). It may prove difficult to identify genetic variations in the ability to maintain maximum photosynthetic rates in the afternoon.

In summary, the results from this study demonstrated that genotypic variation exists in some of the components that make up the photosynthetic system. Unfortunately, because most of the variation identified was associated with poor photosynthetic performance, this study also exemplifies the difficulties that will be encountered when trying to breed for improved photosynthesis. The few variations in components of photosynthesis in the positive direction provide some encouragement that progress might be possible. Nevertheless, the odds of simultaneously breeding for further yield and photosynthetic improvements through conventional techniques seem to be poor at this point. A more thorough screening of the germplasm pool or the use of genetic engineering may be needed to identify or produce a sufficient level of variation in the components needed to increase the odds of successfully breeding for higher photosynthesis and yield.

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